Possible role of SSB protein in the regulation of antibiotic production

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Bacterium *Streptomyces coelicolor* has a complex life cycle that include formation of vegetative mycelium, extrusion of areal hyphae and finally spore formation. This processes require highly regulated cellular differentiation and coordinated gene expression to ensure proper chromosome organization and partitioning of DNA into pre-spore compartments before septa closure.

Single-strand DNA-binding (SSB) proteins have a key role in all processes of DNA metabolism. They act as recruitment platform for many genome maintenance proteins, and while doing so, they modulate their activities. Although many bacteria have additional copies of *ssb* genes, their biological role is poorly understood. *Streptomyces coelicolor* has two paralogous SSB proteins SsbA and SsbB (Fig.1). Our group previously found that SsbA is essential while SsbB participates in chromosomal segregation during sporulation [1].

Our studies showed that paralogous SSB proteins possibly make interactions with various regulatory proteins. Potential interactants were found with Tandem affinity purification (TAP) and Bacterial two-hybrid (B2H) method (Table 1).

Literature data show that the acidic C-tip of SSB protein is important for interactions with other proteins [2], as well as for ssDNA binding [3]. In order to analyze whether (or how) the altered C-terminus of paralogous SSB proteins will interfere their function, we performed various mutations in C-terminus of SsbA and SsbB proteins (Fig. 5). Binding affinity of wild type and various mutants for ssDNA was assessed with isotermal titration calorimetry (ITC). The results of ITC method at low and high salt are shown in the Table 2.

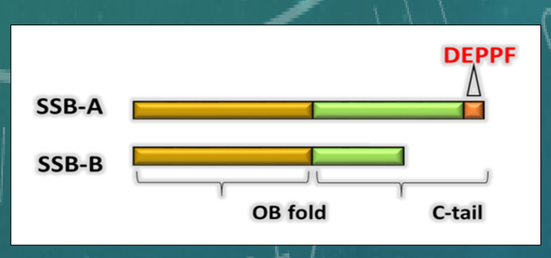

Fig. 2. Differences in the domain structure of paralogous proteins SsbA and SsbB.

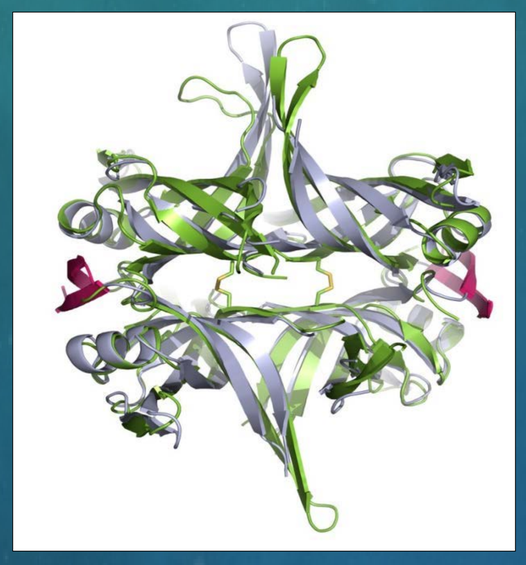

Fig. 1. 3D structures of SsbB (green) and SsbA (grey). Clamp structure of SsbA (pink colour) is functionally replaced by S-S bridges in SsbB tetramer (yellow sticks) [1].

Table 1. Results of TAP and B2H systems.

SsbA – (**B2H+ TAP**) : ∑ **64** potential candidates B2H ∑ **43** potential candidates TAP	Total	**SsbB** – (**B2H**) : ∑ **14** potential candidates	Total
DNA metabolism (RecG*, RecA*, RecQ*, TopoI*...)	**26**	DNA metabolism	**3**
Regulatory (PadR, TetR, MerR, GntR, LuxR, LysR...)	**43**	**Regulatory** (LysR, GntR, TCA...)	**5**

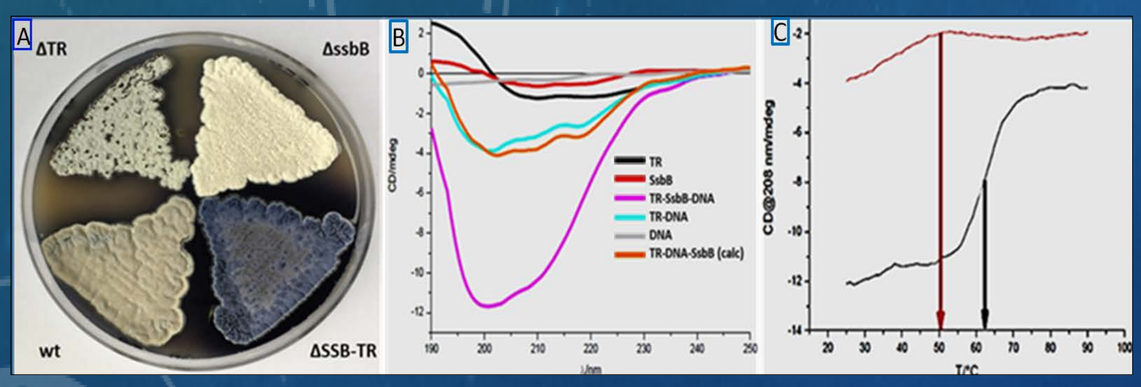

Fig. 3. A - Deletion of GntR; TR-increased Act production; B - CD spectra indicated interactions of SsbB and GntR-TR; C - showed increased stability of the complex.

Conserved motifs: **DEPPF** , **PXXP**, **GGX**

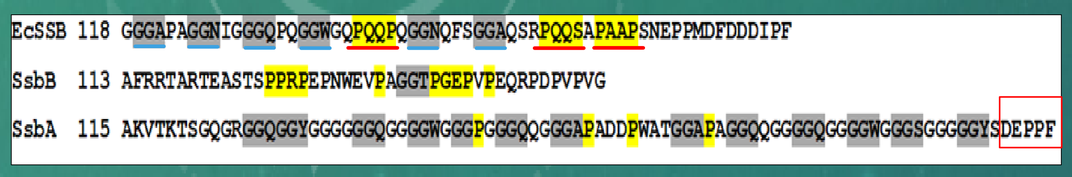

Fig. 4. C-domains of paralogous SSBs comprise different conserved motifs.

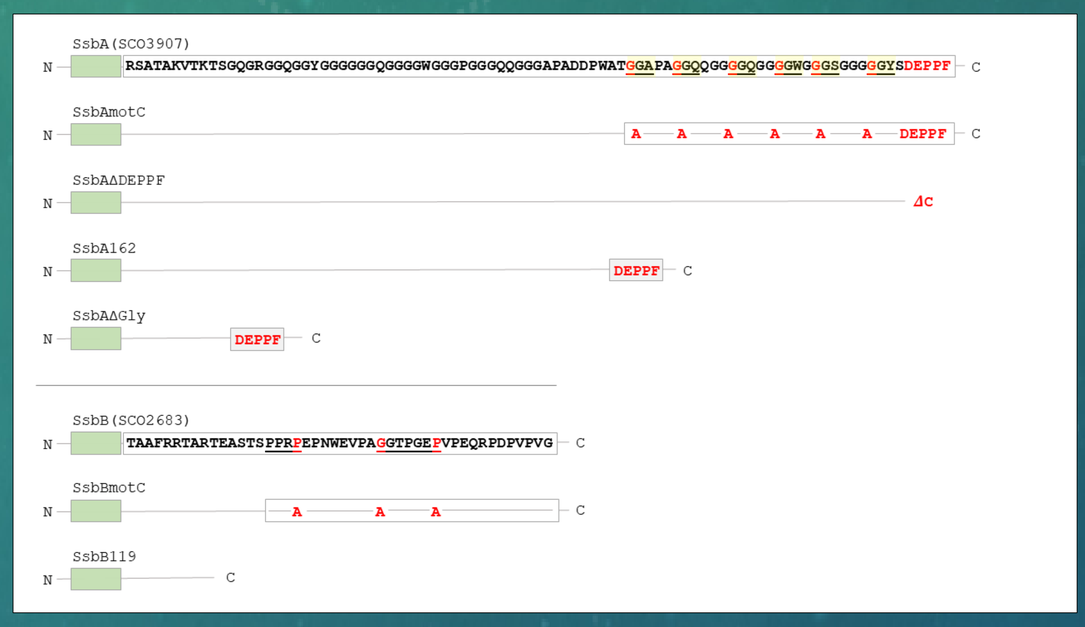

Fig. 5. Constructs of SsbA, SsbB and their mutants.

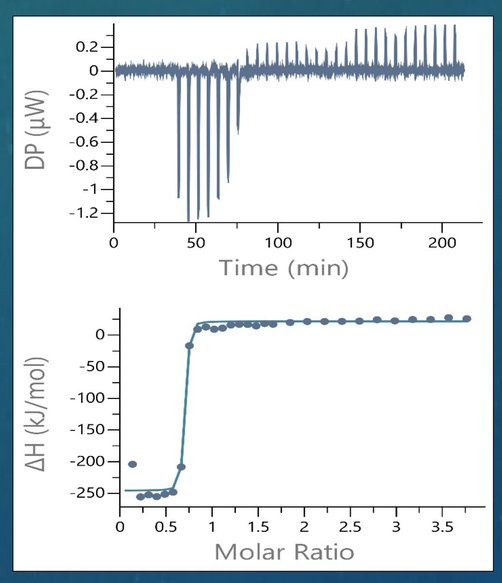

Fig. 6. ITC spectra of DNA binding to SsbA protein.

Table 2. Dissociation constans obtained with ITC measurements at low and high salts.

Protein	**dT**	**I_c/mM**	**K_d**	**I_c/mM**	**K_d**
SsbA	45	30	3,55E-10	300	1,71E-07
dDEPPF	45	30	1,47E-09	300	1,20E-07
A162	45	30	2,37E-10	300	1,39E-07
dGly	45	30	6,01E-10	300	7,08E-07
SsbAmutC	45	30	7,15E-10	300	2,95E-08
Protein	**dT**	**I_c/mM**	**K_d**	**I_c/mM**	**K_d**
SsbB	45	30	6,59E-10	300	6,43E-07
SsbBmutC	45	30	1,21E-10	300	6,56E-08

- Double mutation of ssbB and TR (member of GntR-like transcriptional regulator family) increased the synthesis of the antibiotic actinorhodin (Fig 3.A). This suggests that SsbB might be directly or indirectly involved in the regulation of secondary metabolite production by stabilization of TR/DNA complex.
- Mutations of conserved motifs found in C-terminal domains of SsbA and SsbB proteins affect the binding affinity for ssDNA.
- Deletion of conserved motifs in SsbA result in higher binding affinity at low salt than the wt SsbA, but in high salt both ssbA and ssbB with mutated conserved motifs show higher binding affinity than wild types, suggesting that not only the lenght, but the amino acid composition is important for DNA binding.
- Since the same motifs might be involved in SSB protein interactions, same mutations will be used to examine protein-protein interactions between paralogous SSB proteins and their possible interactants found by B2H and TAP technology.

References:

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